Brain Pathology 8: 175-193(1998)

Cellular Pathology of Lysosomal Storage Disorders

Steven U. Walkley

Sidney Weisner Laboratory of Genetic Neurological Disease, Department of Neuroscience
Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx (NY), USA

Lysosomal storage disorders are rare, inborn errors of metabolism characterized by intralysosomal accumulation of unmetabolized compounds. The brain is commonly a central focus of the disease process and children and animals affected by these disorders often exhibit progressively severe neurological abnormalities. Although most storage diseases result from loss of activity of a single enzyme responsible for a single catabolic step in a single organelle, the lysosome, the overall features of the resulting disease belies this simple beginning. These are enormously complex disorders with metabolic and functional consequences that go far beyond the lysosome and impact both soma-dendritic and axonal domains of neurons in highly neuron type-specific ways. Cellular pathological changes include growth of ectopic dendrites and new synaptic connections and formation of enlargements in axons far distant from the lysosomal defect. Other storage diseases exhibit neuron death, also occurring in a cell-selective manner. The functional links between known molecular genetic and enzyme defects and changes in neuronal integrity remain largely unknown. Future studies on the biology of lysosomal storage diseases affecting the brain can be anticipated to provide insights not only into these pathogenic mechanisms, but also into the role of lysosomes and related organelles in normal neuron function.

Corresponding author:
Steven U. Walkley, DVM, PhD, Sidney Weisner Laboratory of Genetic Neurological Disease, Dept. of Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, (NY) 10461, USA, Tel.:718/430 4025; Fax:718/430 8821; E-mail: walkley@aecom.yu.edu

Introduction

This year marks a quarter century since the appearance of the landmark publication "Lysosomes and Storage Diseases" (24) in which Hers and Van Hoof and their colleagues presented in full detail their treatise on the then relatively new field of lysosomal disorders. A mere eight years before Hers had proposed, based on his studies of glycogenosis (23), that so-called "storage diseases" were actually caused by deficient activity of lysosomal enzymes (46). According to this view, absence of hydrolytic activity of an individual lysosomal enzyme would result in a catabolic defect and lead to accumulation of a specific metabolic product normally cleaved by that enzyme. At the time most of the known storage diseases were recognized both by their clinical characteristics and by accumulation (hence the term "storage") of a major type of substrate (gangliosides, mucopolysaccharides, etc.). During the period following Hers' proposal the study of storage diseases expanded rapidly and a great number of these diseases were confirmed as being "lysosomal" in origin. These included a variety of enigmatic disorders that had been identified earlier in the century (or before), like Tay-Sachs, Hurler, Niemann-Pick, Krabbe and Gaucher diseases, as well as newer disorders like fucosidosis and mannosidosis. Clearly what had emerged was an explanation for an entire family of disorders believed linked by genetic defects involving individual enzymes in a single organelle, the lysosome. The resulting metabolic blockade and lysosomal storage appeared to be key events responsible for cellular dysfunction and the clinical disease exhibited by the individual. Thus, from gene defect to enzyme deficiency, to intracellular storage and functional compromise of cells, these diseases fulfilled Garrod's classic concept of inborn errors of metabolism (15) as well as any known genetic diseases. There was even optimism for the possible successful treatment of these diseases based on delivery of the missing enzyme to the lysosomal system (10).

Successful delineation of individual storage diseases according to enzyme deficiency and substrate storage has been followed in more recent years by important advances in understanding the molecular genetics of these disorders (42, 63). This explosion of information

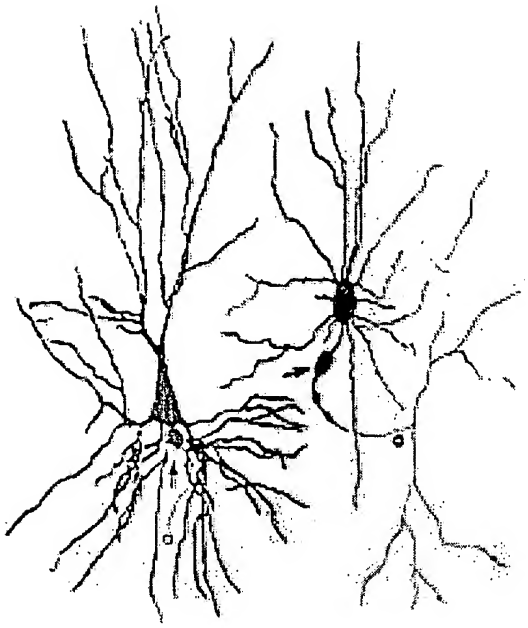

Figure 1. Two of the most conspicuous and well documented examples of morphological alterations that neurons undergo in storage diseases are ectopic dendritogenesis and axonal spheroid formation, yet the link between the primary lysosomal event and these cellular changes is little understood. The illustration above shows two cortical neurons from feline GM2 gangliosidosis. The pyramidal neuron on the left exhibits ectopic dendritogenesis at the axon hillock as it would be seen in a Golgi impregnation (91). The nonpyramidal GABAergic neuron on the right exhibits axonal spheroid formation as would be seen following an intracellular peroxidase injection (33). Immunocytochemical studies have documented the massive abundance of these spheroids within axons of GABAergic neurons (81). Ectopic dendritogenesis does not occur on GABAergic neurons, and axonal spheroids are at best only very rarely seen affecting cortical pyramidal neurons in storage diseases. The conundrum is how does a single lysosomal hydrolase deficiency cause two such diverse events involving both dendrites and axons?

about specific gene defects generated a more complex picture of the overall nature of this family of disorders, compared to the original, simpler view of a single enzyme deficiency being responsible for an individual storage disease. That is, most lysosomal enzyme defects have been found to be caused by multiple gene mutations. For example, over 50 mutations are now identified as affecting the α-subunit of the β-hexosaminidase enzyme, deficiency of which is linked to Tay-Sachs disease and its variants (20). Advances in molecular genetics of storage diseases have also tended to overshadow analysis of events at the other end of the disease spectrum, namely those unanswered questions concerning the important issue of disease process. Specific genetic mutations may be well characterized as causing defective function of individual lysosomal enzymes, but how the resulting metabolic changes then generate the complex events occurring in cells and tissues, particularly brain, remains poorly understood. Neurons are also known to exhibit diverse and often bizarre alterations in morphology secondary to the storage disease process, and may do so in a highly neuron-type specific fashion. For example, some types of neurons in widely diverse kinds of storage diseases exhibit what clearly is one of the most unusual and unprecedented changes of any neurological disease, the growth of new, primary dendrites (Figure 1A). Likewise, other neurons, again in a cell-selective manner, undergo changes in axons, focal swellings or spheroids, at sites far removed from that of the primary lysosomal derangement (Figure 1B). There is every reason to believe that these changes in neuronal morphology have profound consequences for neuron and brain function yet we know little about the link between the lysosomal derangement and the generation of these neuron-specific alterations. In other storage disorders, particularly the Batten family of storage diseases, it is the death of neurons rather than changes in neuronal morphology per se that appears to cause brain dysfunction. This loss of neurons is again a highly cell type specific phenomenon, even though intraneuronal storage is universal, and the reason why particular neurons die is unknown. The purpose of this review is to explore and reexamine some of these unresolved issues involving the pathogenesis of lysosomal diseases, issues of disease process residing at the level of individual organelles and cells, particularly the neuron. It is, after all, the neuron, largely due to its longevity, that provides perhaps the widest array of questions and greatest importance in these disease states. Overall descriptions of the cellular pathology of individual lysosomal storage diseases can be found in excellent recent reviews (e.g., see 35).

The Lysosome and Storage

In his chapter on lysosomes in the above-mentioned text by Hers and Van Hoof (24), Novikoff summarized the then current view of the lysosomal system in normal cells (46). His figure illustrating the lysosomal system (reproduced here as Figure 2) reveals the primary lysosome, filled with a complement of lysosomal hydrolases, as fusing with a variety of other vesicular organelles, including autophagic and digestive vacuoles and multivesicular bodies. These primary lysosomes were believed to have shuttled from the Golgi apparatus

to endocytic vacuoles where fusion occurred, thus forming secondary lysosomes. Residual bodies, viewed as vacuoles containing undigested materials, membrane fragments, myelin figures, etc., were found in normal cells to a degree, but also were recognized as the hallmark of the so-called lysosomal storage diseases. Terry and colleagues carried out the first electron microscopic analysis of storage bodies in Tay-Sachs disease in 1963 and showed the presence of conspicuous multilamellated organelles which they referred to as membranous cytoplasmic bodies or mcb's (70). Similarly, and in the same year, Batten disease neurons were distinguished from those in Tay-Sachs as they contained a radically different, amorphous and "fingerprint" material (98). This discovery finally set Batten disease apart from Tay-Sachs disease and led to the coining of the term "neuronal ceroid lipofuscinosis" to define the former. Within a short time, characteristic storage bodies or "cytosomes" were identified for a host of different types of storage diseases. Indeed, it appeared that major classes of storage diseases could be distinguished by the ultrastructural appearance of their storage material: Glycosphingolipidoses presenting with membranous swirls (mcb's), mucopolysaccharidoses with multilamellar stacks ("zebra bodies"), fucosidosis and mannosidosis with watery or wispy material ("open" inclusions), and so forth (67). The explanation for the characteristic appearances of storage bodies was that cell material entered the lysosome in normal fashion but once there the lack of a specific degradative enzyme led to a buildup of individual metabolic products. Accumulation of this material past a certain critical point was believed to lead to physicochemical interactions with other materials within the lysosome (e.g., cholesterol) and to formation of the characteristic morphological appearance of the residual or storage body for a given disease. Numerous studies even in the early 1960's showed that residual bodies in storage diseases like Tay-Sachs and other neurolipidoses contained acid phosphatase activity suggesting a lysosomal status. Arguments that the storage bodies sometimes lacked histochemical evidence of lysosomal enzymes, or even lysosomal-like delimiting membranes, and thus might not be lysosomal, were eventually interpreted as simply late disease-associated alterations in the lysosome, rather than arguments against the lysosomal disease concept.

Remarkable advances have occurred in our understanding of the lysosome since Novikoff's early summary (29). We now recognize over 40 different enzymes within lysosomes, including glycosidases, lipases, phospholipases, phosphatases, sulfatases, and proteases, and genetic deficiencies and related storage diseases have been identified for most of these (42, 63). We now understand that lysosomal storage in storage diseases may occur secondary to one of several mechanisms: That is, a particular lysosomal enzyme may be absent as a result of a lack of production of mRNA or to unstable mRNA for the enzyme. The mRNA that is present may produce an enzyme of abnormal structure that is less effective in cleaving the appropriate substrate(s). Alternatively, the functional enzyme deficiency may be due to processing or trafficking errors. In these cases, lysosomal enzymes generated in the endoplasmic reticulum or Golgi apparatus may fail to undergo normal post-translational modification leading again to either absent or diminished activity. There may also be abnormal recognition signals leading to errors in trafficking and to misrouting or inappropriate secretion. These latter two groups of processing abnormalities can involve a host of enzymes simultaneously, as is documented in multiple sulfatase deficiency and in I-cell disease. Some lysosomal enzymes are known to require co-factors or protective proteins and their absence has also been linked to defects in lysosomal enzyme function. Lysosomal membrane receptors may themselves be abnormal leading to abnormalities in substrate entry or exit, as is the case in sialic acid storage disease, also known as Salla disease. Finally, several types of storage diseases, most notably Niemann-Pick disease type C and selected forms of Batten disease, stand apart from these well-defined primary lysosomal defects. Current understanding suggests that they result from essentially nonlysosomal events involving trafficking of other types of molecules which secondarily impact the lysosomal system (48, 51).

The relationship between genotype and phenotype at the level of disease process remains poorly defined for most storage disorders. It is known that the specific genetic event precipitating the enzyme deficiency can have profound consequences for disease progression and clinical presentation. These include both the degree of storage and the brain regions most severely affected. Generally speaking, the relative activity of an affected lysosomal enzyme in a given storage disease appears to correlate with the age of onset and severity of disease (9), with most storage diseases appearing as infantile, late infantile, juvenile, or adult-onset variants (63). The existence of compound heterozygotes in which patients possess two dissimilar mutant alleles has been documented to lead to highly unusual disease variants as seen, for example, with GM2 gangliosidosis (20).

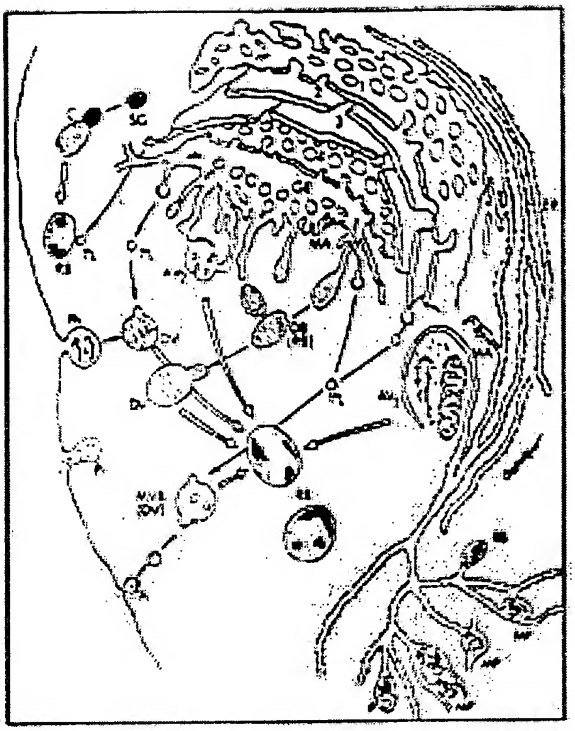

Figure 2. An illustration from Novikoff (46) showing the relationship between lysosomes and other organelles at the time of the development of the lysosomal storage disease concept. (1°L, primary lysosome; AV, autophagic vacuoles; C, crinophagy; DB, dense body; DV, digestive vacuole; ER, endoplasmic reticulum; GE, Golgi; MA, microautophagy; MP, microperoxisome; MVB, multivesicular body; P, peroxisome; Ph, phagocytic vacuole; Pi, pinocytic vacuole; RB, residual body; SG, secretory granule.) (From 46, with permission)

Storage disease-related effects also have been identified in phenotypically normal heterozygotes, as has been reported in a feline model of Niemann-Pick disease type C (7). In this case, occasional cortical neurons exhibited ganglioside storage in a manner very similar to diseased cells in homozygous affected individuals. Non-CNS abnormalities were also detected.

The Endosomal-Lysosomal System. Materials are known to be delivered to lysosomes by multiple pathways, including those associated with endocytosis, autophagy, and phagocytosis. One of the best studied of these pathways relative to the lysosome is the endosome. Indeed, an important advance in the modern understanding of the lysosome as an organelle relates to its important functional association with endosomes and the concept of the endosomal-lysosomal system (1, 34, 43). This association is based in part on the finding that lysosomal enzymes are localized to endosomal compartments, most notably late endosomes, and that the pH of these organelles is acidified (pH 5-6), though not as much so as lysosomes themselves (pH 4-5). There are currently two dominant hypotheses that attempt to explain the relationship between endosomes and lysosomes. The "maturation" model evolved from earlier views of the lysosomal system as summarized in Novikoff's diagram (Figure 2). That is, secondary lysosomes (shown in the figure as digestive vacuoles) are formed following fusion of primary lysosomes containing newly synthesized lysosomal enzymes from the Golgi apparatus, and endosomes (referred to as phagocytic or pinocytic vacuoles in this same diagram). Thus, according to this view, the early endosome gradually matures into a late endosome and, following fusion with primary lysosomes, its functional status is gradually converted to that of a lysosome. A second hypothesis, the so-called "stable compartment" model, argues that early and late endosomes are separate stable populations of organelles. Significant differences in pH, in the presence of specific rab proteins, and in mannose-6-phosphate receptors and lysosomal hydrolases are supportive of the latter model. These models have been widely discussed and compared (3, 21, 40, 49).

A more complete understanding of the endosomal-lysosomal system likely will provide important insights into many of the major unanswered questions involving pathogenesis of certain types of lysosomal storage diseases. That is, viewing storage diseases as wholly lysosomal phenomena and the lysosome as a mere end-organelle for catabolic processing provides little insight into pathogenic mechanisms. If catabolic enzyme defects lead simply to lysosomal storage and no "extra-lysosomal" consequences, one must almost necessarily predict an outcome of simple cell death secondary to cell swelling to account for the neurological dysfunction that characterizes these diseases. Indeed, simple "cytotoxicity" following mechanical disruption of cells has historically, and often, been invoked as a sort of shorthand explanation for cell dysfunction in lysosomal diseases. But the cell-selective consequences of the storage disease process mentioned earlier (and as described in detail below) argue strongly that significant extra-lysosomal events are set in motion by deficiencies of single lysosomal enzymes. How might these occur? At least four possible mechanisms that reach beyond the mere mechanical disruption hypothesis can be cited: (i) Material not catabolized within the lysosome may escape this organelle and in turn have deleterious effects elsewhere in the cell. These effects could be directly toxic or they could impact other metabolic pathways and

alter cell function. (ii) The massive accumulation of unprocessed compounds within the lysosome (e.g., gangliosides) may deprive the cell of certain precursor molecules, leading to a compensatory upregulation or dysfunction of other metabolic pathways or organelles (e.g., see 62). (iii) Abnormalities of the lysosomal system may lead to a reduced entry of materials into lysosomes and to subsequent increases of this compound elsewhere in the cell, with detrimental consequences. (iv) Defective lysosomal enzyme activity may adversely effect the normal functioning of other related organelles, most notably endosomes.

Few of the above possibilities have been rigorously tested for individual types of storage diseases. Perhaps the most intriguing of these is the latter one based on the close association between lysosomes and the endosomal system, and the potential role of lysosomal enzymes within endosomes. If, in storage diseases, both the endosomal and lysosomal compartments are actively involved in the "storage" process, disruption of a variety of signal transduction and other events could be envisaged that could account for the dramatic changes induced in neurons secondary to the enzyme defect. Whereas the lysosome is still most often regarded as a site of end-stage processing for macromolecules, the endosome is viewed as a much more dynamic player in cellular events. Endosomes have been documented as being central to the internalization and processing of cell surface receptors and to playing a role in transmembrane signaling (3). However, not only are many receptors known to be internalized via endosomes, but gangliosides and other cell surface constituents destined for lysosomal degradation are also believed to follow similar trafficking (60). Indeed, some types of membrane receptors that cycle by way of endosomes have been implicated as having functional relationships with specific types of gangliosides. For example, EGF receptors, reportedly associated with GM3 ganglioside (5), accumulate in coated pits after binding EGF and subsequently appear in early and late endosomes, and eventually are degraded in lysosomes. However, evidence is emerging that the internalization process is not simply a mechanism of deactivation of this receptor. Instead, there appears to be both continuation and augmentation of EGF receptor activity within endosomes, including receptor tyrosine phosphorylation which exceeds that attained by the plasmalemmal EGF receptor population (74). Numerous studies have linked modulation of EGF receptor function with GM3 ganglioside, whereby GM3 appears to act to inhibit the formation of EGF receptor dimers after stimulation of cells with EGF (5). Further,

Disease/Neuron	With Ectopic Dendritogenesis	Without Ectopic Dendritogenesis
Types of *Neuronal Storage Diseases:*	- GM1 Gangliosidosis - GM2 Gangliosidosis - Niemann-Pick disease type A - Niemann-Pick disease type C - Mucopolysaccharidosis type I - a-Mannosidosis	Batten disease Fucosidosis
Types of *Neurons:*	- Cortical Pyramidal Neurons - Multipolar Neurons of the Amygdala and Claustrum - Granule Cells of the Fascia Dentata - Spiny Neurons of the Striatum	- Cortical Nonpyramidal Neurons - Motoneurons of the Brain Stem and Spinal Cord - Principal Relay Neurons of Thalamus - Purkinje Cells and other Cerebellar Neurons

Table 1. Examples of neurons and neuronal storage diseases with and without ectopic dendritogenesis. The same types of neurons in each disease are vulnerable to dendritic sprouting.

gangliosides and other glycosphingolipids have shown positive or negative modulatory effects not only on EGF receptors, but also on receptors for NGF, FGF, PDGF, p60c-src and insulin (5, 8, 13, 41, 53, 95). Interestingly, ligands for all of these receptors, as well as cholera toxin (a ligand for GM1 ganglioside), have been implicated in endosomal signal transduction pathways (3).

Effects on Neurons

It has long been appreciated that neurons in storage diseases not only exhibit somatic swelling secondary to the abnormal accumulation of material, but also reveal a variety of other somatic, dendritic and axonal changes. In the earliest papers by Sachs and colleagues on cases of what was then referred to as amaurotic family idiocy (now called Tay-Sachs disease), neurons were described as being swollen and filled with abnormal material (55, 56). Particular note was made of enlargements within the basilar dendrites of diseased cortical neurons and contrast was drawn to spinal motoneurons which exhibited swelling but no dendritic changes. Thus an appreciation of the concept of neuron type-specific changes came early to the study of neuronal storage diseases. In later years so-called axonal swellings were also noted in this and other storage diseases, and terms like "axonal torpedoes", "torpedo bodies" and "axonal spheroids" were variously applied. Use of these often ill-defined terms, coupled with basic misunderstandings as to specific structures actually involved, led to erroneous notions about the cellular pathology of many lysosomal storage diseases.

The difficulty inherent in an analysis of the cellular pathology of storage diseases is aptly demonstrated in a

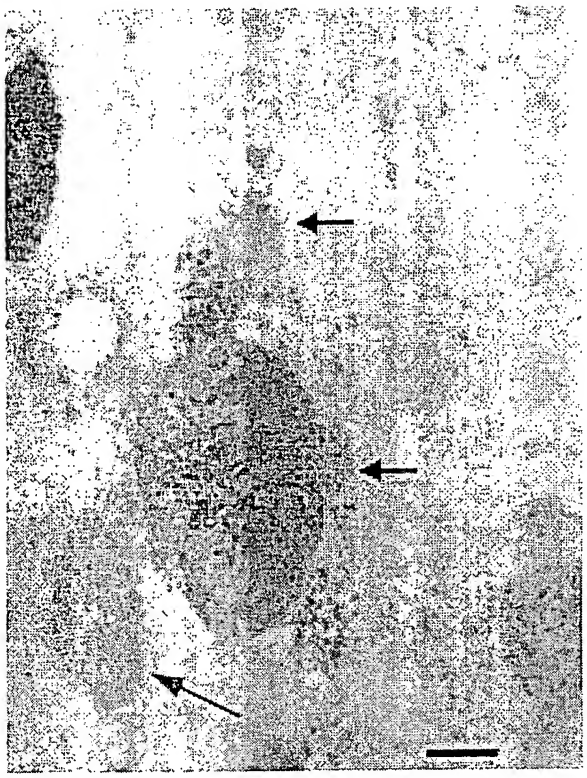

Figure 3. PAS stain of a 2 μm thick plastic section of enlarged neurons in the cerebral cortex from a cat with GM1 gangliosidosis. The massive lysosomal system storage can be seen as PAS-positive perikarya and meganeurites (long arrow). Smaller spherical structures (short arrows) are PAS negative and are axonal spheroids. Calibration bar equals 15 μm.

simple PAS stain of cerebral cortex in a case of ganglioside storage disease (Figure 3). It is readily evident that the neuron at the center of the field is filled with an abnormal accumulation of PAS-reactive material and that the nucleus is displaced into the base of the apical dendrite. Another PAS-positive torpedo-shaped structure is nearby which lacks a nucleus. Structures similar to the latter were observed by Sachs and Strauss (57) in their early studies of Tay-Sachs disease and they are now known to represent parasomatic swellings located at the axon hillock of neurons, as will be described below. Also seen in Figure 3 are two barely visible spherical structures that do not contain PAS-reactive material, one sitting immediately adjacent to the perikaryon, the other in the nearby neuropil. We now recognize these structures to be axonal in nature, so-called axonal spheroids, and to be distinctly different from the other cellular enlargements.

There was considerable appreciation for the existence of unusual swellings on Tay-Sachs disease-affected pyramidal neurons in many early studies (most notably see Bielschowsky, ref. 4). However, an accurate definition of the true nature of these changes had to await the application of modern principles of neurobiology coupled with, ironically, a time-honored staining method dating to the days when storage diseases were first discovered, the Golgi technique (52). Beginning in 1976, Purpura and colleagues demonstrated unequivocally that the parasomatic enlargements of Tay-Sachs disease were not enlargements of basilar dendrites but were of axon hillock origin (see Figure 4). The finding was of crucial importance because it indicated that this enlargement consisted of "new" dendritic-like membrane rather than simply being an enlargement of an existing (basilar) dendrite. This discovery also clearly distinguished dendritic changes from axonal changes and argued against the use of ill-defined terms, like torpedo bodies, to characterize such structural abnormalites. Thus two distinct types of cellular alterations emerged as characteristic of many storage diseases, namely meganeurites and axonal spheroids.

Meganeurite formation in storage diseases. According to the early studies of Purpura and colleagues, meganeurites were parasomatic enlargements within the axon hillock and thus were proximal to the initial segment of the axon (52, 85). They appeared to occur secondary to storage, i.e. as part of a volume expansion by the neuron, since they always contained massive numbers of storage cytosomes identical to those found in the rest of the perikaryon. They were found only on certain types of neurons while other types, just as Sachs had reported, appeared to undergo simple somatic enlargement secondary to storage. Although originally described as being composed of dendritic-like membrane in diseases like Tay-Sachs, subsequent studies of other storage diseases revealed that there were really two classes of meganeurites (75, 76). Some were "spiny" and covered with dendritic-like spines, neuritic extensions and synapses and therefore appeared to be composed of dendritic-like membrane. Others were "aspiny" and lacked any evidence of dendritic spines or new synapse formation. Detailed studies of animal models of ganglioside storage diseases further revealed that both spiny and aspiny meganeurites could occur on specific populations of neurons, whereas in other diseases, like Batten disease (84, 93), only aspiny meganeurites were found (Figure 5). The issue was further complicated by the presence of some neurons exhibiting growth of ectopic dendritic membrane in the form of synapse-covered neuritic sprouts in the absence of a meganeurite. An

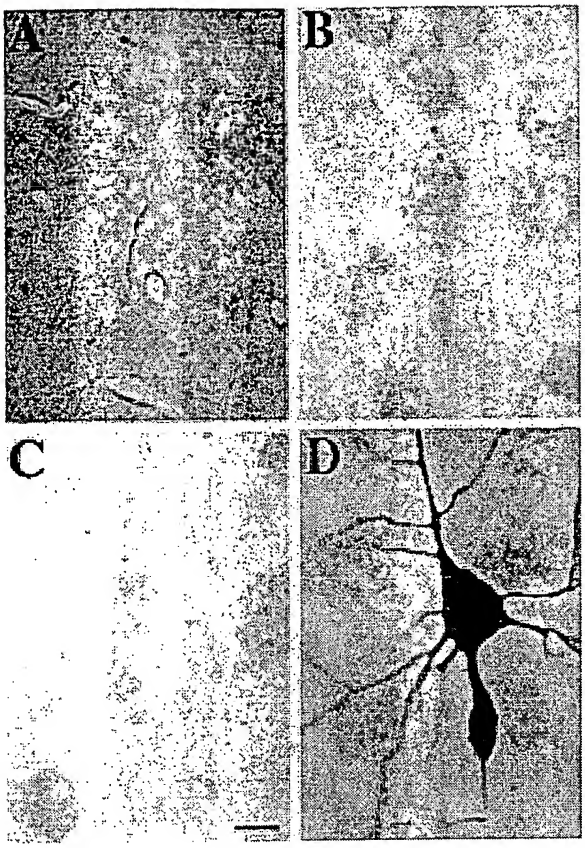

Figure 4. Studies using a histochemical (ferric ion - ferrocyanide staining) technique to identify the axonal initial segment have shown that meganeurites occur proximal to this region and spheroids distal to this region (85). **A.** normal neurons with dark staining at the initial axonal segment; **B.** layer III pyramidal neuron with a meganeurite with initial segment staining distal to this structure; **C.** nonpyramidal-like neuron with an axonal spheroid and initial segment staining between the perkaryon and the spheroid. **D.** layer III neuron with a meganeurite as visualized with a Golgi stain. (**A-C**, 2 μm plastic sections counterstained with safranin red. **D**, Golgi stain). Calibration bar equals 18 μm.

example of this type of neuron is shown in Figure 1A. Such ectopic neurite-bearing neurons, however, were commonly associated with other neurons of the same type that demonstrated the presence of spiny meganeurites. Thus what emerged was the view that specific types of neurons in certain storage diseases appeared capable of undergoing elaboration of new dendritic membrane at the axon hillock, with this new membrane occurring either as neuritic sprouts or as spiny meganeurites (or both). Other storage disorders, like Batten disease, had pyramidal and other neurons with aspiny meganeurites only. In these cases, the meganeurite was viewed exclusively as a volume-accommodation event

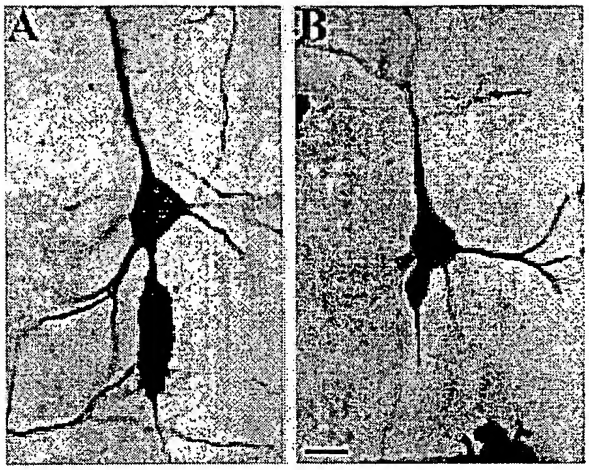

Figure 5. Comparison of pyramidal neurons with a "spiny" meganeurite as seen in feline GM1 gangliosidosis (A) and an "aspiny" meganeurite as seen in canine Batten disease (B). The former represents new, synapse-covered dendritic-like membrane, whereas the latter is simply a volume increase in the cell, as described in the text. Golgi stains. Calibration bar equals 15 μm.

since there was no evidence of new dendritic membrane or new synapses being present. As shown in Figures 6-7 and summarized in Table 1, a variety of types of neurons and storage diseases have been shown to exhibit ectopic dendritogenesis, whereas other cell types and diseases lack this change. An important finding in these studies was that the same types of neurons in all diseases characterized by ectopic dendritogenesis consistently demonstrated the capacity to undergo new dendrite growth. That is, only a few select types of neurons appeared capable of undergoing this process, and these were always the same, even in metabolically diverse storage disorders (75, 76, 78).

Ectopic dendritogenesis and neuronal storage. The discovery that pyramidal neurons in Tay-Sachs disease undergo regrowth of new dendritic membrane in the form of spiny meganeurites and secondary neuritic processes led Purpura and Suzuki to propose two key hypotheses: Firstly, they argued that these structures and their related aberrant synaptic inputs occurring proximal to the axonal initial segment could be responsible for some aspects of brain dysfunction in these diseases. Secondly, they proposed that gangliosides (the primary storage material in this disease) might in some way be linked to the regrowth of new dendritic membrane. It was subsequently discovered that ectopic dendritogenesis occurred in both ganglioside and non-ganglioside storage diseases and the same types of neurons were

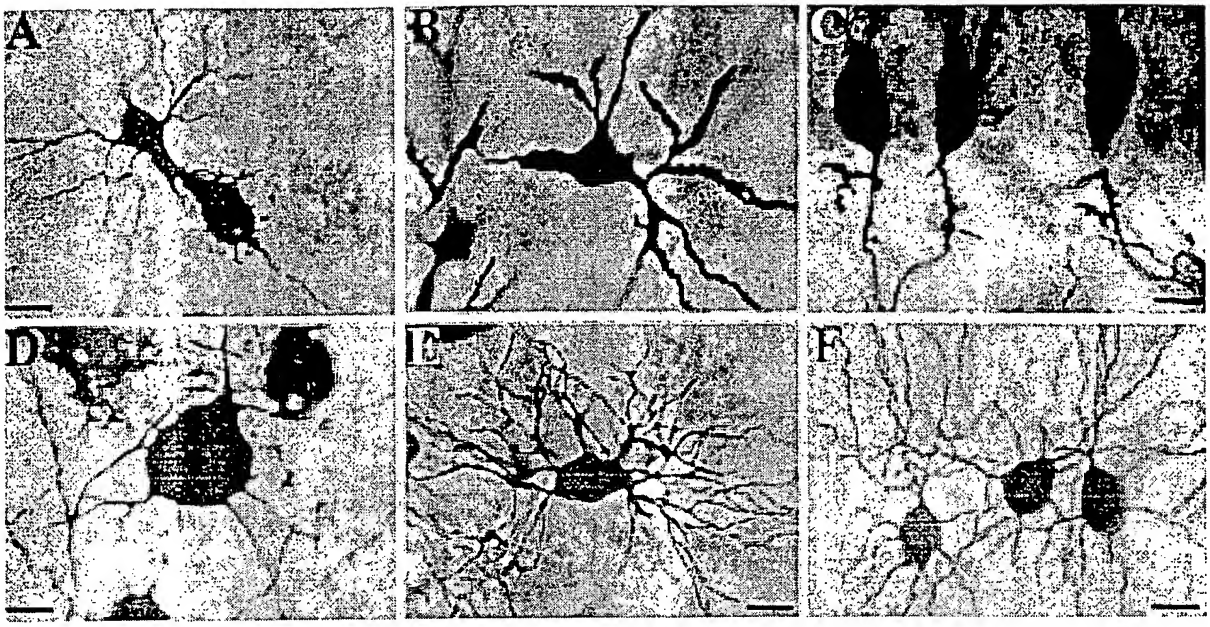

Figure 6. Golgi impregnations of neurons in feline GM1 gangliosidosis showing types of cells susceptible to new dendrite growth. Spiny meganeurites were observed on multipolar cells of the claustrum (A) and caudate nucleus (B). Ectopic dendritic membrane was observed sprouting from the axon hillock region of occasional granule cells of the fascia dentata (cells that normally lack basilar dendrites) (C). Cells that lacked meganeurites or ectopic dendrites included motoneurons (D, oculomotor nucleus), principle neurons of thalamic relay nuclei (E, nucleus ventralis basalis) and nonpyramidal neurons of cerebral cortex (F). Golgi stains. Calibration bars equal A (also for B) 30 μm; C, 15 μm; D, 30 μm; E, 25 μm; F, 20 μm.

consistently affected (79, 80, 82, 83, 88, 91) (Figures 6-7) (Table 1). It could be argued that the presence of ectopic dendritogenesis on the same types of neurons across diverse types of storage diseases indicates the importance of some intrinsic feature unique to these particular neurons. The de novo sprouting of new primary dendrites is a highly unusual event and has not been reported to occur under other circumstances. An obvious question therefore was whether the neurons with ectopic dendrites had specific characteristics in common. Although all such neurons possessed dendritic spines, other dendritic spine-possessing neurons (e.g., Purkinje cells) did not sprout new dendrites. Likewise, axon projection status, neurotransmitter-type, and other features also were not unique to neurons with ectopic dendrites. It is true that the three classes of cells most conspicuously involved with ectopic dendritogenesis, cortical pyramidal neurons and multipolar cells of the amygdala and claustrum, are telencephalic-derived neurons of similar pyramidal-like morphology, but no other common features are evident in terms of cell type.

A consideration of the wide spectrum of storage diseases characterized by ectopic dendritogenesis likewise reveals no common metabolic link (Table 1). And since some types of storage diseases lacked the phenomenon, lysosomal compromise itself appeared an unlikely explanation. Closer examination of one particular kind of storage disease, α-mannosidosis, did, however, provide a crucial clue. This disease was found to exhibit ectopic dendritogenesis in a very limited fashion with only 15-20% of pyramidal cells being affected (although all neurons showed storage) (18, 88). In analyzing neurite-bearing cortical pyramidal neurons in this disease it was readily discovered that these cells consistently contained membranous storage material (Figure 8). This was in clear contrast to adjacent pyramidal neurons lacking ectopic dendrites as they contained only typical wispy or clear inclusions. This remarkable contrast in storage material between neurons was also found to be readily apparent in PAS and toluidine blue-stained 2 μm plastic sections of cerebral cortex. This finding not only illustrated the unique vulnerability of individual neurons to the secondary consequences of the primary metabolic defect, but also suggested that glyco-lipid storage was a feature of neurite-bearing pyramidal neurons in α-mannosidosis. Viewing this discovery within the wider context of all other types of storage diseases exhibiting ectopic dendritogenesis suggested that the common metabolic change underlying the sprouting might be storage of a particular glycolipid, most likely a ganglio-

side. Through a series of studies this fact was indeed established, with GM2 ganglioside shown to be the one metabolic product consistently elevated in the cerebral cortex of all storage diseases characterized by ectopic dendritogenesis (64, 87). By immunocytochemistry, GM2 ganglioside was further localized to vesicular structures in the cytoplasm of ectopic dendrite-bearing pyramidal neurons (77) (Figure 9). Whether all such vesicular structures are actually tertiary lysosomes is not yet established, nor is it yet known why elevations in GM2 ganglioside occur in pyramidal neurons in so many types of storage diseases (apart from GM2 gangliosidosis). However, it is known that the elevations in GM2 ganglioside precede the appearance of ectopic dendritic sprouting (18) and that ectopic dendritogenesis is most common in GM2 gangliosidosis (64). These findings indicate that the increases in GM2 are likely a cause, and not a consequence, of ectopic dendritogenesis (87).

It has also recently been shown that normal developing cortical neurons undergoing dendritogenesis also express GM2 ganglioside in a punctate, vesicular pattern, with the GM2-immunoreactivity disappearing as dendritic maturation progresses (19). The identity of these vesicular structures remains to be determined, but their placement near the Golgi apparatus and in nearby cytoplasm is consistent with current views on ganglioside synthesis and trafficking: Gangliosides are synthesized by a series of glycosyltransferases located in the Golgi apparatus, and after synthesis, they are believed to be translocated to the plasmalemma by vesicular membrane flow (58, 59, 61, 62, 72, 73, 96). Evidence suggests that some types of gangliosides may undergo axonal transport (e.g., see 25), whereas others are transported to the soma-dendritic domain. Exocytic transport vacuoles budding from the Golgi apparatus are believed to move to the plasmalemma where membrane fusion results in ganglioside being inserted with their hydrophilic head groups on the surface of the neuron. Here, current understanding is that gangliosides have the potential to move in the plane of the membrane and to interact with a variety of membrane receptors and other proteins on which they may have modulatory effects (22, 26, 36, 62, 95, 97). Endocytosis and subsequent recycling of gangliosides may involve internalization via coated pits, followed by endosomal transport to lysosomes (61, 62).

The simplest explanation for why GM2 ganglioside is elevated in storage diseases other than GM2 gangliosidosis is that it is due to a generalized lysosomal derangement that impacts function of its catabolic

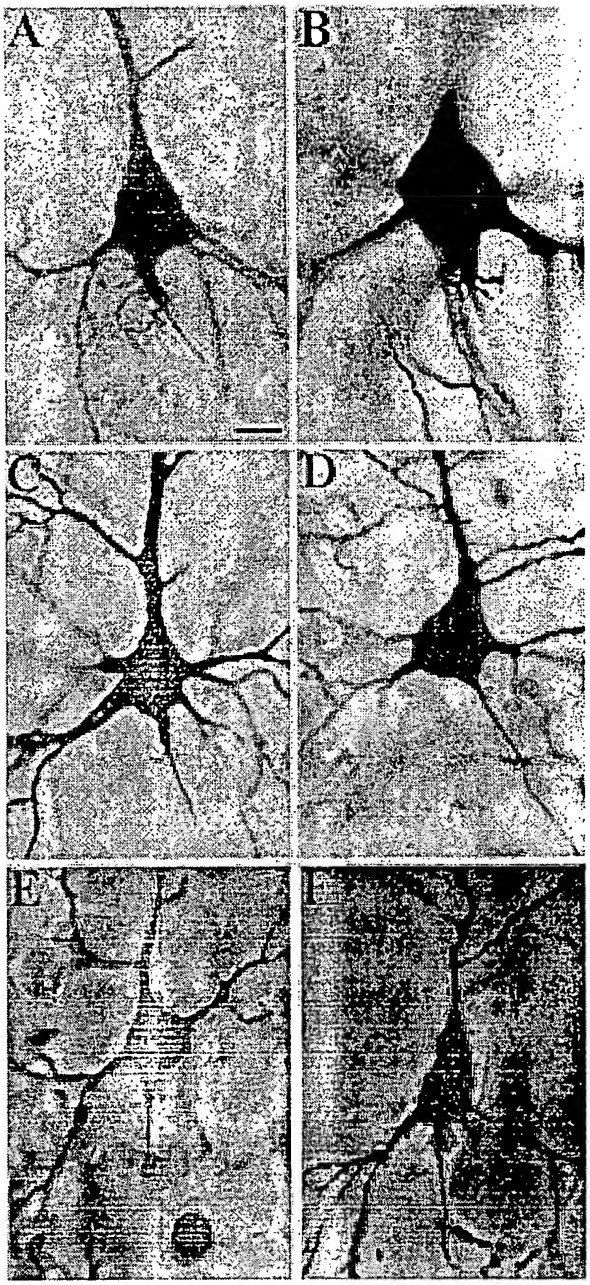

Figure 7. Examples of ectopic dendrite growth on cortical pyramidal neurons in a variety of lysosomal storage disorders in feline models. **A.** GM1 gangliosidosis; **B.** swainsonine-induced α-mannosidosis; **C.** mucopolysaccharidosis type I; **D.** Niemann-Pick disease type A; **E.** GM2 gangliosidosis; **F.** Niemann-Pick disease type C. Golgi stains. Calibration bar equals 18 μm.

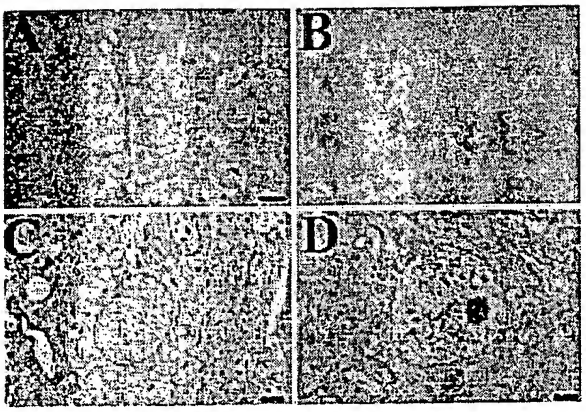

Figure 8. Studies examining the nature of the storage material in cortical neurons in feline α-mannosidosis revealed adjacent neurons with radically different types of storage. (**A**) toluidine blue (TB) stained 2 μm plastic section showing diversity of staining of storage material. Neuron on the far right exhibits TB-positive storage, the others are negative. (**B**) Similarly, PAS staining reveals occasional positive cells (on right) whereas most cells exhibit storage, but no staining. **C** and **D**, electron micrographs of neurons in the cerebral cortex showing typical clear, open inclusion apparent in most cells (**C**) as compared to those less frequent neurons containing cytosomes with membranous storage material (**D**). Calibration bars equal 10 μm for **A** and **B**; **C**, 4 μm; **D**, 3 μm.

enzyme, β-hexosaminidase. Yet there is no evidence suggesting a consistent and specific defect in the activity of this enzyme in these disparate storage diseases. Similarly, it could be conjectured that changes in ganglioside synthesis have occurred, but this issue has not been systematically studied in storage diseases. In normal developing neurons there is no reason to assume that the GM2-immunoreactive organelles are exclusively lysosomes. Rather, based on current knowledge of ganglioside synthesis and trafficking, exocytic vacuoles and endosomes are the likely organelles involved. Therefore it is not clear how the expression of an individual ganglioside in such diverse conditions, mature neurons undergoing lysosomal storage and normal, immature neurons undergoing dendritic differentiation, could be linked to regulation of dendritic sprouting. Clearly the suggestion is that there may be a common location of GM2 ganglioside in both circumstances. In normal neurons, as mentioned earlier, current hypotheses suggest that gangliosides function by modulating activity of growth factor or other receptors located in the plasmalemma. Both receptors and gangliosides are believed to enter the cell via endosomal trafficking prior to lysosomal degradation. Thus, the plasmalemmal-endosomal interface appears a likely common site where GM2 may function to influence receptors, which

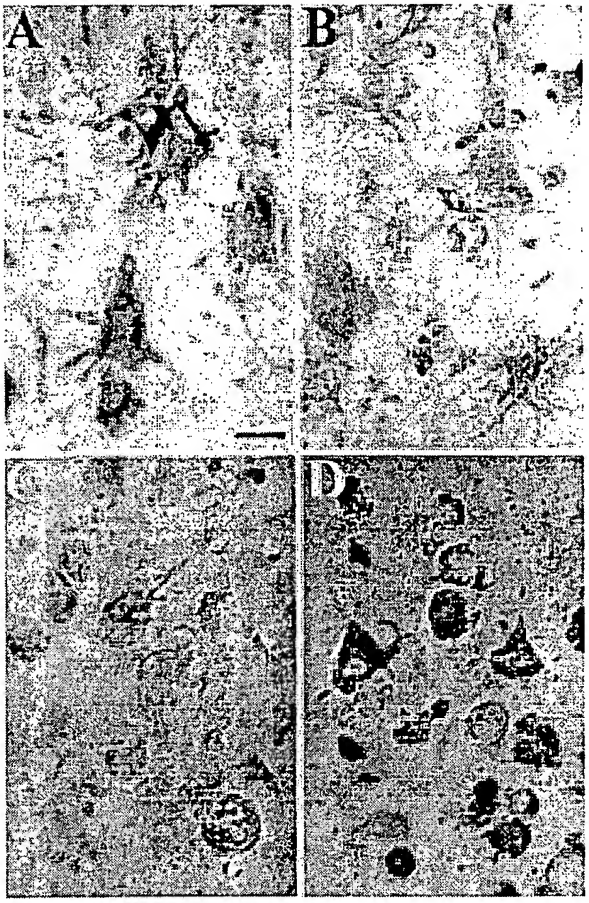

Figure 9. Immunocytochemical studies to localize GM2 ganglioside in storage diseases reveals that it is present in cortical pyramidal cells when these neurons exhibit ectopic dendritogenesis based on Golgi staining (**A.** α-mannosidosis; **C.** mucopolysaccharidosis type I; **D.** Niemann-Pick disease type C). Disease lacking ectopic dendrite growth on the pyramidal cell population also lack evidence of GM2-immunoreactivity in these cells although GM2 elevations may be seen in glial cells (**B.** fucosidosis). PAP immunocytochemistry with TB counterstain; Calibration bar equals 20 μm.

in turn through interactions with second messengers, could lead to dendritic outgrowth. There are also other indications that the plasmalemma in ganglioside storage disease neurons is not normal (90). Fine gold labelling using a Golgi-EM method has revealed numerous membrane redundancies (Figure 10) resembling "ruffles" and other cell surface phenomena believed associated in other cell types with macropinocytosis, a form of endocytosis (69).

The above findings suggest that during active dendritogenesis GM2 ganglioside may be associated with the endosomal system in both storage disease-affected and

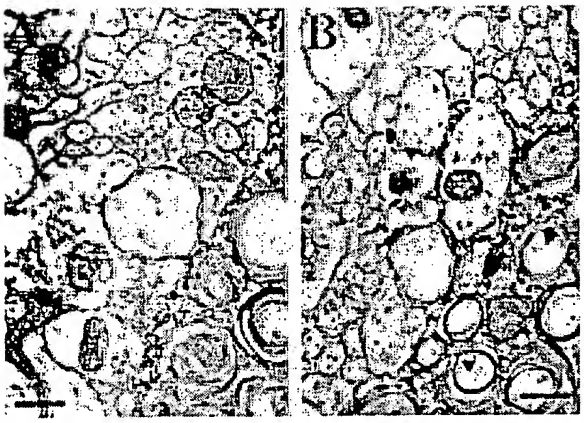

Figure 10. Combined Golgi-EM analysis of cortical neurons in a feline model of ganglioside storage disease reveals the presence of membrane "ruffles" on the surface of the neuron. Gold particles replace the silver chromate of the Golgi reaction and label thin plasmalemmal extensions. Calibration bars equal for **A**, 0.3 μm; **B**, 0.4 μm.

normal developing neurons. This is of particular importance since endosomes, as discussed earlier, have been documented as playing a role in the internalization and processing of cell surface receptors as well as in transmembrane signaling. These studies offer the provocative suggestion that gangliosides may exercise their modulatory influence over cell signaling specifically at the plasmalemmal-endosomal interface. Understanding the precise localization of GM2 ganglioside during dendritic sprouting in storage diseases is clearly of central importance in unraveling its role in this phenomenon.

Axonal spheroid formation in storage diseases. Just as meganeurites are structural enlargements proximal to the initial segment of the axon, axonal spheroids are enlargements distal to this region (Figure 4). There is also a second critical distinction: Whereas meganeurites contain storage material consistent with the specific defective lysosomal hydrolase, axonal spheroids contain a distinctly different array of materials with ultrastructural features that are the same across many types of storage disorders. That is, they consist of tubulovesicular profiles, dense bodies, mitochondria, and related materials. Most importantly, they do not contain cytosomes equivalent to those observed in the perikaryon or meganeurite for a given type of storage disease.

For many years axonal spheroids were presumed to be "nonspecific" phenomena potentially occurring in all types of neurons. However, in studies of animal models of storage diseases using immunocytochemical methods it has been determined that the vast majority of the

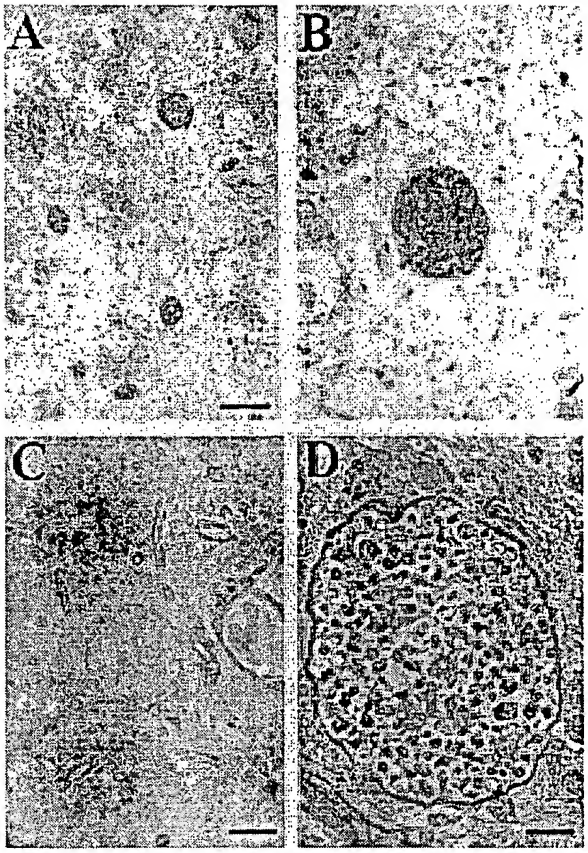

Figure 11. Studies of axonal spheroids in the cerebral cortex of feline models of lysosomal diseases reveal that they predominate in GABAergic neurons. Immunocytochemical staining for GAD, the synthetic enzyme for GABA, identifies spheroids within axons of this type of neuron (**A**, **B**). In routine plastic sections without immunostaining spheroids are more difficult to identify (**C**). EM analysis of spheroids (**D**) reveal a typical ultrastructure that is similar across brain regions and types of storage diseases. Calibration bars equal **A**, 20 μm; **C** (and **B**) 5 μm; **D**, 1.2 μm.

spheroids can be labeled with antibodies specific for GABAergic cell populations, namely GAD (glutamic acid decarboxylase) and calcium binding proteins (parvalbumin, calretinin, calbindin) (38, 81). Indeed, this immunocytochemical approach to identifying spheroids has also shown that they are far more abundant in gray matter areas, like cerebral cortex, than previously appreciated (Figure 11). Antibodies to specific cytoskeletal elements like neurofilaments or microtubule associated proteins only rarely label spheroids (Figure 13). This finding is consistent with ultrastructural evidence showing a lack of accumulation of such cytoskeletal elements within spheroids. Immunocytochemical and other studies, including direct neuronal injection studies (Figure

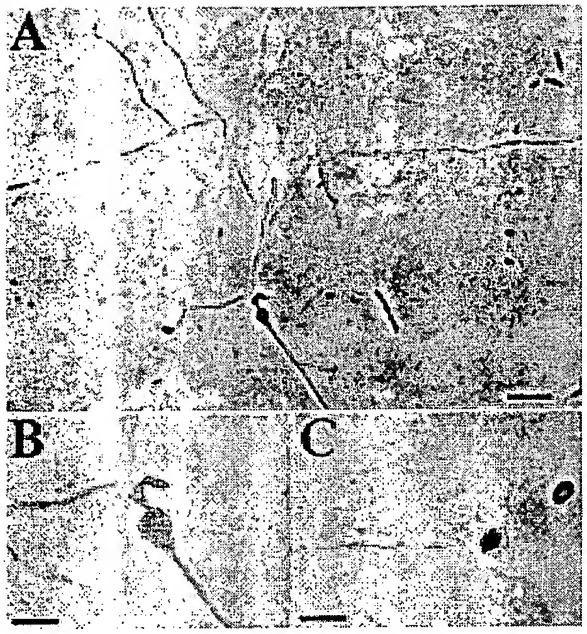

Figure 12. Studies in which GABAergic cells were directly injected with horseradish peroxidase show morphology of axons and spheroids in detail. **A.** Axonal field and partial view of cell body of a putative GABAergic neuron (based on somadendritic morphology); **B.** Spheroid in mainstem dendrite appears flatter on proximal side suggestive of retrograde filling; **C.** Smaller spheroids were also visible in the axonal terminal fields of this cell. Calibration bars **A**, 40 μm; **B**, 20 μm; **C**, 15 μm.

12) (33), have also shown that spheroids occur in axons at a variety of sites. A given axon may have numerous spheroids. These may occur proximally and near the cell body (but distal to the axonal initial segment), at various sites along the length of an individual axon, or in the terminal axonal field (including within synapses).

Axonal spheroids in storage diseases do not appear to be "retraction bulbs" of dying axons (Figures 12-13). Rather, they are swellings of significant size that occur along the length of an axon with axonal continuities clearly visible on both proximal and distal sides. These structural characteristics of spheroids suggest that they may be caused by defects in axoplasmic transport. Accumulations of similar heterogeneous organelles have been reported to occur distal to crush or low temperature lesions in axons, indicating that these types of materials are characteristic of a block in retrograde transport (50, 66, 71). The close similarity of accumulated material in the lesion studies to that of spheroids in storage diseases suggests that the latter may also be secondary to a block in retrograde transport. One type of neuron most susceptible to spheroid formation, the cerebellar Purkinje cell, also is a cell type that is highly vulnerable to cell death in storage diseases. In this case cell death follows spheroid formation and may be secondary to it. That is, spheroid formation and a block in retrograde movement of a growth factor or other element essential to the survival of this class of neuron might be responsible for their selective death. Presently there is less evidence in support of a defect in anterograde transport to explain the formation of spheroids. For example, in GABAergic neurons, the anterogradely-transported enzyme, glutamic acid decarboxylase (GAD), fills the spheroid (hence allowing their identification, see 81), but GAD-immunoreactivity in synaptic terminals of these cells appears to be normal.

In attempting to address the question as to why a defect in axoplasmic transport might occur in a wide variety of primary lysosomal diseases, one logical possibility is that the lysosomal system compromise in perikarya simply deprives the axon of a key component required for the normal movement of organelles or other material. This could involve, for example, either deficiencies of lysosomes or lysosomal hydrolases, the ability to acidify lysosomes, or key components of molecular motor transport mechanisms. As for lysosomes, their possible occurrence in axons has received remarkably little direct examination. Retrogradely-carried endosomes, e.g. from synaptic terminals, are generally believed to fuse with lysosomes only after endosomes have reached the neuronal cell body (for discussion see refs. 28, 43). However, some older (and recent) research findings do provide substantial support for the presence of at least small numbers of lysosomes and lysosomal enzymes in axons (6, 16, 17, 30, 47). If lysosomes are transported down axons even in small numbers they may fuse with retrogradely transported endosomes with resulting catabolic processing being initiated well before endosomes reach perikarya. Massive involvement of the lysosomal system may deprive axons of this lysosomal input and result in endosomal traffic without lysosomal fusion, i.e. enlarged endosomes. Indeed, the enlarged tubulo- and multi- vesicular profiles seen in spheroids may in fact be enlarged endosomes.

A second possibility is that lysosomal compromise in perikarya might deprive the axon of a key transport molecule such as kinesin or dynein. Indeed, cytoplasmic dynein, the retrograde molecular motor, has been shown to be associated with perikaryal lysosomes and other organelles (37, 44, 49). Finally, another obvious question that deserves consideration: Why are GABAergic neurons particularly vulnerable to axonal spheroid formation? It is widely believed that many types of

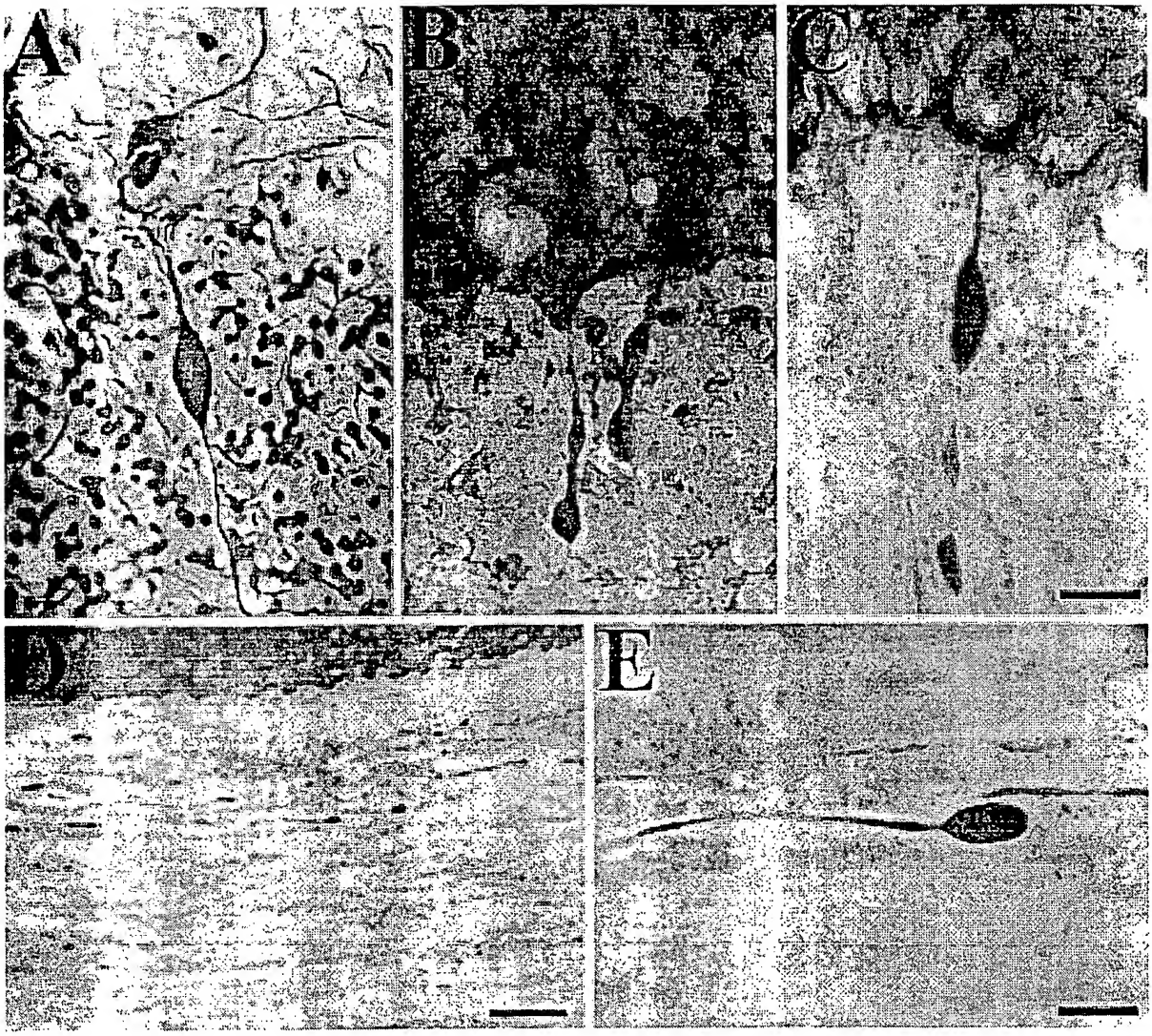

Figure 13. Purkinje cells in storage disease are particularly prone to axonal spheroid formation. As occurs in other types of neurons, spheroids may be seen in proximal (cerebellar cortex, **A-C**) and distal (cerebellar white matter, **D-E**) areas of axons. **A.** Bodian stain; **B.** GAD immunocytochemistry; **C.** neurofilament 68 immunocytochemistry; **D.-E.** GAD immunocytochemistry. Note continuation of distal axon past spheroid in E. Calibration bars equal for **C** (and **A, B**) 35μm; **D**, 110 μm; **E**, 30 μm.

GABAergic neurons have higher firing rates and metabolic activity than other types of neurons (e.g., see 32). This feature may result in a greater turnover in axonal and synaptosomal components, and thus greater reliance on fully intact anterograde and/or retrograde transport mechanisms.

Spheroids observed in the studies reported above generally have not exceeded 20-30 μm in diameter but even this size likely is capable of causing significant interference with the efficacy or timing of action potential propagation (81). Given recent developments in understanding the crucial role of inhibition in sculpting neuronal activity, such abnormalities would be anticipated to have profound effects on brain function. Indeed, one of the most compelling findings to emerge from these immunocytochemical studies using animal models of GM1 and GM2 gangliosidosis, Niemann-Pick disease, α-mannosidosis, and mucopolysaccharidosis, is the striking correlation between the location and incidence of axonal spheroids and the type and severity of clinical neurological disease (38, 81). Of these diseases only MPS I lacks both significant neuro-

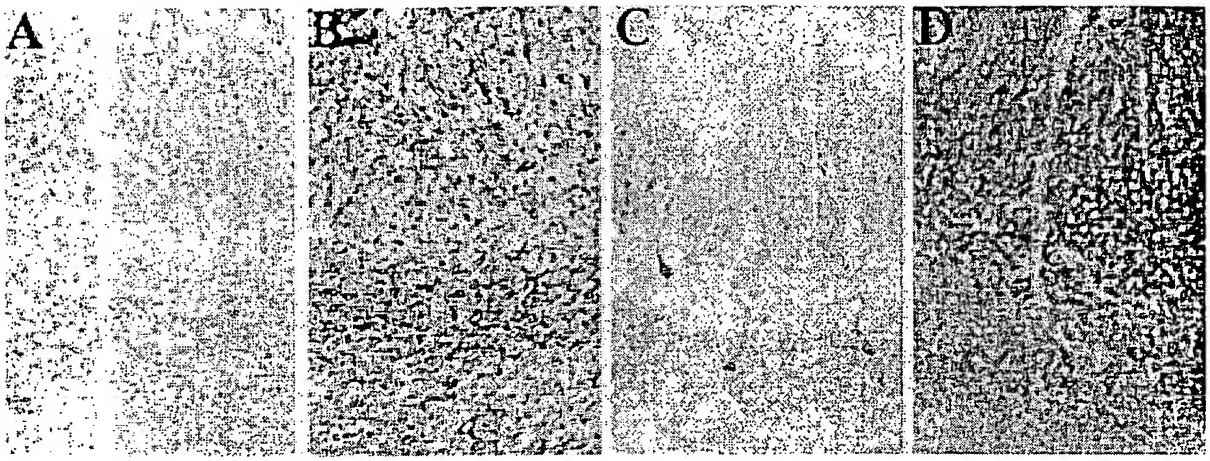

Figure 14. Studies of cerebral cortex in canine NCL show that cortical changes predominate in layer IV. **A.** Nissl stain for overall cellularity; **B.** GFAP stain for astrocytic reactions; **C.** SMI-32 stain for cortical pyramidal neurons; **D.** MAP2 stain for dendrites. Overall there appears to be increased gliosis and neuron loss in the layer IV area. Cortical depths in the 4 sections are matched. All figures shown at 65x magnification.

logical disease and evidence for significant axonal spheroid formation. This is in spite of widespread (near universal) neuronal storage and, indeed, even the presence of spiny meganeurites and ectopic dendritogenesis/synaptogenesis affecting cortical neurons. For the other storage diseases analyzed, the degree of motor system deficits and other neurological findings fully correlate with the incidence of spheroids within the GABAergic cell populations in specific brain regions. It is highly likely, based on these studies, that axonal spheroids account for the generation of more of the clinical neurological disease in these animal models than any other known abnormality, including intraneuronal storage, meganeurite formation and ectopic connectivity and neuron death.

Neuron death. By end stage disease, neuronal cell loss is observed in many types of lysosomal storage disorders. Certain types of neurons, as described for Purkinje cells above, may be vulnerable to cell death earlier in some storage diseases. However, massive death of neurons and accompanying brain atrophy is not a regular feature of the early stages of most types of storage diseases, with one significant exception. This is the disease family known as neuronal ceroid lipofuscinosis or Batten disease. These diseases have been grouped together largely on the basis of similarities in the ultrastructural appearance of the storage material which appears as so-called "curvilinear" or "fingerprint" bodies, or variants thereof. Like other storage disorders, the NCL diseases also occur as infantile, late infantile, juvenile and adult-onset forms. An inability to identify any specific lysosomal hydrolase deficiencies in these diseases, or even to clearly define the storage material (other than as ceroid-lipofuscin), led to these disorders literally being left behind by advances in understanding of most other types of storage disorders in the 1970' and 80's. The discovery that a major storage product in affected cells in the late infantile, juvenile and adult forms of NCL was subunit c of mitochondrial ATP synthase led to their re-classification as lysosomal proteinoses (48). Recent advances defining the gene defects for several of these conditions are also consistent with lysosomal protease deficiencies (27, 65).

The NCL family of diseases is characterized by early death of some types of neurons and accompanying atrophy in specific regions of brain. Most conspicuously affected is the cerebral cortex, with the degree of atrophy rivaling that of Alzheimer's and other neurodegenerative diseases. A conspicuous difference from these adult-onset dementias is, of course, that the atrophy is occurring in childhood for most of the Batten-type disorders.

A major unanswered question is why neurons die in the NCL disorders. Storage is widespread and occurs not only in brain but also in a variety of other tissues. Disease-related cell death, however, predominates only in cerebral cortex, and in some disease forms, in the cerebellum and retina. Other brain regions and tissues show no significant cell loss. Interestingly, the changes in neurons described earlier for other types of storage diseases, ectopic dendritogenesis and axonal spheroid formation, are absent in Batten disease. As shown earlier (Figure 5) some pyramidal neurons do exhibit mega-

neurites but these lack evidence of dendritic features and GM2 ganglioside is not a component of the storage process. Likewise, GABAergic synaptic terminals identified by immunocytochemical techniques may be diminished in number in some brain regions like cerebral cortex or cerebellum, but GAD-positive axonal spheroids are not observed (39, 84).

Selective neuronal vulnerability is a common characteristic of most neurodegenerative diseases and, as described above, the NCL disorders are no exception. One recently hypothesized mechanism to account for this phenomenon in Huntington disease and related disorders is chronic glutamate excitotoxicity (2). According to this view, the neurotransmitter glutamate is deleterious to specific neurons either because it is overabundant or because specific neurons have become vulnerable to its effects due to receptor changes or metabolic defects within the cell (45). This latter view, the so-called "weakened target cell" model, is based on impaired energy production within neurons possessing glutamate receptors (31). Thus, suboptimal mitochondrial function in neurons receiving abundant excitatory glutamatergic input would be anticipated to lead to excessive calcium influx, free radical formation, and cell death. A variety of studies of NCL diseases also suggest mitochondrial abnormalities and free radical damage (54). There is also evidence that subunit c of mitochondrial ATP synthase undergoes abnormal trafficking in NCL cells (11, 12). Animal model studies have supplied evidence for compromise of select inhibitory (GABAergic) circuits in cerebral cortex and cerebellum, and loss of inhibitory inputs likely would exacerbate excitotoxic mechanisms (39, 84).

Evidence supporting the hypothesis of chronic excitotoxicity can be found in the pattern of neuron loss in both the cerebral cortex and cerebellum in canine Batten disease. Changes in specific cerebral cortical layers have been evaluated during disease progression using cytochrome oxidase histochemistry, anti-GFAP and anti-SMI32 antibodies (for astroglial and cortical pyramidal cell labelling, respectively). It has been found that an early and consistent feature of cortical atrophy is reduced cytochrome oxidase activity, a marked astrocytic response, and neuron loss, all of which are centered specifically in midlevel cortex between pyramidal cell layers III and V (Figure 14). The affected layer (IV) in normal brain represents the major receiving zone in cerebral cortex for excitatory thalamocortical inputs. This cortical layer normally demonstrates the highest endogenous cytochrome oxidase staining indicative of a normal, high sustained metabolic activity (94). That cell death and gliosis predominate here in the canine NCL cortex is consistent with intrinsic metabolic demand (and therefore mitochondrial function) being an important player in the process of cell death. Additionally, a subpopulation of scattered GABAergic neurons in NCL cortex exhibit massively enlarge mitochondria, suggestive of a direct compromise in these mitochondria or secondarily-induced physiological reaction unique to this type of neuron (39, 84).

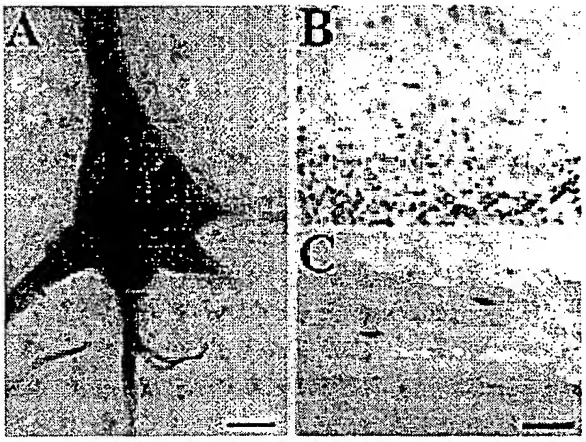

Figure 15. Studies using swainsonine-induced a-mannosidosis reveal that disease-related changes in tissues are not readily reversible post-metabolic correction. **A.** Ectopic dendrites on a Golgi-impregnated cortical pyramdal neuron 2 years after withdrawal of enzyme inhibitor. **B.-C.** Nissl stain of cerebellar cortex revealing loss of Purkinje cells (**B**), and persistence of axonal spheroids (**C**), 4 years post-treatment. Calibration bars equal for A, 10 μm; C (and B) 50 μm.

Similar events also have been reported in the cerebellum in the canine Batten disease model (39). Significant loss of basket cells and basket cell terminals which supply inhibitory inputs to Purkinje cells appears to occur before Purkinje cell loss and cerebellar atrophy. The basket cell population also displays evidence of unique metabolic stress like that of cortical inhibitory neurons: massively enlarged and abnormal-appearing mitochondria. Other neurons exhibit reduced cytochrome oxidase activity.

Reversibility of neuronal changes. An issue of major importance in terms of treating lysosomal storage diseases is that of the potential for reversibility of the cellular pathology characterizing these diseases. In the very early days of the lysosomal disease concept de Duve predicted that correction of these diseases might follow uneventfully if diseased cells had the opportunity and ability to internalize the missing enzyme (10). This is due to the observation that endosomal trafficking

should target the enzyme to the exact organelle, the lysosome, in which it was needed. Numerous studies now demonstrate this capability and a few lysosomal diseases, notably those without CNS involvement, have been effectively treated. Even the neuronal storage disease, α-mannosidosis, has been corrected following bone marrow transplantation (89). In this case it is believed that donor bone marrow-derived microglial cells in brain secrete α-mannosidase which is internalized by neurons with correction of the storage disease.

An issue not addressed in the studies on treatment of genetic studies carried out to date concerns evaluation of the reversibility of cellular changes in brain, most notably ectopic dendritogenesis and axonal spheroid formation. To get at this question an inducible model of neuronal storage has been used, namely that of swainsonine-induced (and reversible) α-mannosidosis (86). Swainsonine is an indolizidine alkaloid derived from locoweed and some other plants. It is a reversible inhibitor of α-mannosidase and if ingested chronically over a 2-4 month period induces a phenotypic replica of genetic α-mannosidosis. Using this model it has been learned that vacuolated lysosomes disappear rapidly (within days) of enzyme replacement (i.e, cessation of administration of the enzyme inhibitor) and neuron swelling and meganeurite formation likewise disappear (92). However, ectopic dendrites and axonal spheroids proved otherwise. Although the incidence of ectopic dendrites was less in animals evaluated months and years after disease correction, some neuritic processes persisted, and indeed appeared to elongate with time in the absence of the disease process (Figure 15). This suggests that once formed, ectopic dendrites may be treated as constituent parts of the neuron as a whole. What makes some ectopic dendrites remain and others disappear is not clear, but stabilization by established synapses is one possibility (78). Likewise, whether such ectopic dendrites and their synaptic input contributes to cortical dysfunction in otherwise metabolically corrected brain remains to be determined.

Axonal spheroids were also found to persist in the swainsonine disease model even years after disease reversal, suggesting that once formed, these structures are not easily eliminated by the neurons even in the face of a normalized perikaryal lysosomal system. Purkinje cell death also appeared to continue after withdrawal of the toxin (Figure 15), possibly due to prolonged deleterious effects of spheroids on these cells, as discussed earlier. In this case the correlation between brain dysfunction and persisting cellular alterations is more apparent. Animals in which storage disease had been induced with swainsonine routinely continued to exhibit cerebellar dysfunction long after complete disappearance of storage from neurons.

Summary

Our knowledge of the genetic and molecular bases of lysosomal storage diseases has expanded dramatically in recent years but a similar indepth understanding of pathogenic mechanisms underlying these diseases has not been achieved. The pathogenic cascade of storage diseases can be exceedingly complex and involve not only secondary lysosomal storage but also a host of nonlysosomal events that may impact the cell in diverse ways. As a consequence, storage diseases are far more complex than their simple origins as single enzyme deficiencies might suggest. As recently argued by Kunihiko Suzuki, one of the pioneers in the study of biochemical and molecular bases of lysosomal diseases (68), the future of studies on storage diseases, to a very large degree, lies in the understanding of their biology. The rewards of these studies will be not only a better grasp on the full range of treatment strategies for these disorders, but too, greater insight into the workings of normal neurons and other cells through study of their kind in the absence of a single enzyme. Garrod's reflection (14) on William Harvey's original view of the study of "rarer forms of disease" is as apt today as three centuries ago. That is, by understanding the details of the pathogenic mechanisms of these diseases, we will learn much about the working of normal cells.

Acknowledgements

I thank M. Zervas and Drs. D. Siegel and K. Dobrenis for numerous helpful discussions and for their collaborative efforts. This work is supported by grants from the NIH and the Ara Parseghian Medical Research Foundation.

References

1. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J (1994) The Molecular Biology of the Cell. 3rd Edition. Garland: New York
2. Beal MF (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative disease? *Ann Neurol* 31: 119-130
3. Bevan AP, Drake PG, Bergeron JJM, Posner BI (1996) Intracellular signal transduction: The role of endosomes. *TEM* 7: 13-21
4. Bielschowsky M (1932) Histopathology of nerve cells. In: Cytology and Cellular Pathology of the Nervous System, Penfield W (ed.), Paul B. Hoeber: New York

5. Bremer E (1994) Glycosphingolipids as effectors of growth and differentiation. *Curr Top Membranes: Cell Lipids* 40: 387-411
6. Broadwell RD, Oliver C, Brightman MW (1980) Neuronal transport of acid hydrolases and peroxidase within the lysosomal system of organelles: Involvement of agranular reticulum-like cisterns. *J Comp Neurol* 190: 519-532
7. Brown D, Thrall MA, Walkley SU, Wurzelmann S, Wenger DA, Allison RW, Just CA (1996) Metabolic abnormalities in feline Niemann-Pick disease type C heterozygotes. *J Inher Metab Dis* 19: 319-330
8. Chakraborty M, Anderson GM, Chakraborty A, Chatterjee D (1993) Accumulation of high level of pp60 c-srcN is an early event during GM3 -antibody mediated differentiation of neuro-2a neuroblastoma cells. *Brain Res* 625: 197-202
9. Conzelmann E, Sandhoff K (1983/84) Partial enzyme deficiencies: residual activities and development of neurological disorders. *Dev Neurosci* 6: 58-71
10. de Duve C (1964) From cytases to lysosomes. *Fed Proc* 23: 1045-1049
11. Ezaki J, Wolfe LS, Ishido K, Kominami E (1995) Abnormal degradative pathway of mitochondrial ATP synthase subunit c in late infantile neuronal ceroid lipofuscinosis (Batten disease). *Am J Med Genet* 57: 254-259
12. Ezaki JR, Wolfe LS, Kominami E (1996) Specific delay in the degradation of mitochondrial ATP synthase subunit c in late infantile neuronal ceroid-lipofuscinosis is derived from cellular proteolytic dysfunction rather than structural alteration of subunit c. *J Neurochem* 67: 1677-1687
13. Ferrari G, Anderson BL, Stephens RM, Kaplan DR, Greene LA (1995) Prevention of apoptotic neuronal death by GM1 ganglioside. *J Biol Chem* 270: 3074-3080
14. Garrod A (1928) The lessons of rare maladies. *Lancet* 1: 1055-1059
15. Garrod AE (1909) Inborn Errors of Metabolism. Oxford University Press: Oxford
16. Gatzinsky KP, Berthold C-H (1990) Lysosomal activity at nodes of Ranvier during retrograde axonal transport of horseradish peroxidase in alpha-motor neurons of the cat. *J Neurocytol* 20: 989-1002
17. Gatzinsky KP, Berthold C-H, Corneliuson O (1988) Acid phosphatase activity at nodes of Ranvier in alpha-motor and dorsal root ganglion neurons of the cat. *J Neurocytol* 17: 531-544
18. Goodman LA, Livingston PO, Walkley SU (1991) Ectopic dendrites occur only on cortical pyramidal cells containing elevated GM2 ganglioside in α-mannosidosis. *Proc Natl Acad Sci USA* 88: 11330-11334
19. Goodman LA, Walkley SU (1996) GM2 ganglioside is associated with cortical neurons undergoing active dendritogenesis during normal brain development. *Dev Brain Res* 93: 162-171
20. Gravel RA, Clarke JTR, Kaback MM, Mahuran D, Sandhoff K and Suzuki K (1995) The GM2 gangliosides. In: The Metabolic and Molecular Bases of Inherited Disease, Scriver CR, Beaudet AL, Sly WS, Valle D (eds.), Chapter 92, pp. 2839-2879, McGraw-Hill: New York
21. Griffiths G, Gruenberg J (1991) The arguments for preexisting early and late endosomes. *Trends Cell Biol* 1: 5-9
22. Hakomori S (1995) Role of gangliosides in transmembrane signaling and cell recognition. In: Biology of the Sialic Acids, Rosenberg A (ed.), pp. 243-259, Plenum Press Publishers: New York
23. Hers HG (1965) Progress in gastroenterology: Inborn lysosomal diseases. *Gastroenterology* 48: 625-633
24. Hers HG and Van Hoof F (1973) Lysosomes and Storage Diseases. Academic Press: New York
25. Hirschberg, K Zisling R, van Echten-Deckert, G, Futerman AH (1996) Ganglioside synthesis during the development of neuronal polarity. *J Biol Chem* 271 :14876-14882
26. Hoekstra D, Kok JW (1992) Trafficking of glycosphingolipids in eukaryotic cells; sorting and recycling of lipids. *Biochim Biophys Acta* 1113: 277-294
27. Hofmann SL, Lee LA, Lu J-Y, Verkruyse LA (1997) Palmitoyl-protein thioesterase and the molecular pathogenesis of infantile neuronal ceroid lipofuscinosis. *Neuropediatrics* 28: 27-30
28. Hollenbeck PJ (1993) Products of endocytosis and autophagy are retrieved from axons by regulated retrograde organelle transport. *J Cell Biol* 121: 305-315
29. Holtzman E. (1989) Lysosomes. Plenum Press: New York
30. Holtzman E, Novikoff AB (1965) Lysosomes in the rat sciatic nerve following crush. *J Cell Biol* 27: 651-668
31. Horowski R, Wachtel H, Turski L, Löschmann P-A (1994) Glutamate excitotoxicity as a possible pathogenetic mechanism in chronic neurodegeneration. In: Neurodegenerative Diseases, Calne DB (ed.), Chapter 11, pp. 163-175, WB Saunders: Philadelphia
32. Houser CR, Vaughn JE, Hendry SHC, Jones EG, Peters A (1984) GABA neurons in the cerebral cortex. In: Cerebral Cortex: Functional Properties of Cortical Cells, Jones EG, Peter A (eds), Volume 2, pp. 63-89, Plenum Press: New York
33. Karabelas AB , Walkley SU (1985) Altered patterns of evoked synaptic activity in cortical pyramidal neurons in feline ganglioside storage disease. *Brain Res* 339: 329-336
34. Kornfeld S, Mellman I (1989) The biogenesis of lysosomes. *Annu Rev Cell Biol* 5: 483-526
35. Lake B (1997) Lysosomal and peroxisomal disorders. Greenfield's Neuropathology, Graham DI, Lantos PL (eds.) Volume 1, Chapter 11, pp. 657-753, Arnold: London
36. Ledeen RW (1985) Biology of gangliosides: Neuritogenic and neuronotrophic properties. In: Neurobiology of Gangliosides,Gorio A, Haber B (eds.), pp. 147-159, Alan R. Liss, Inc. Publisher: New York
37. Lin SXH, Collins CA (1992) Immunolocalization of cytoplasmic dynein to lysosomes in cultured cells. *J Cell Sci* 101: 125-137
38. March PA, Thrall MA, Wurzelmann S, Brown D, Walkley SU (1997) Dendritic and axonal abnormalities in feline Niemann-Pick disease type C. *Acta Neuropathol (Berl)* 94: 164-172

39. March PA, Walkley SU, Wurzelmann S (1995) Morphological alterations in neocortical and cerebellar GABAergic neurons in canine Batten's disease. *Am J Med Genet* 57: 204-212
40. Murphy RF (1991) Maturation models for endosome and lysosome biogenesis. *Trends Cell Biol* 1: 77-82
41. Mutoh T, Tokuda A, Miyadai T, Hamaguchi M, Fujiki N (1995) Ganglioside GM1 binds to the TRK protein and regulates receptor function. *Proc Natl Acad Sci USA* 92: 5087-5091
42. Neufeld E (1991) Lysosomal storage diseases. *Annu Rev Biochem* 60: 257-280
43. Nixon RA, Cataldo AM (1995) The endosomal-lysosomal system of neurons: new roles. *Trends Neurosci* 18: 489-496
44. Noda Y, Nakata T, Hirokawa N (1993) Localization of dynamin: Widespread distribution in mature neurons and association with membranous organelles. *Neuroscience* 55: 113-127
45. Novelli A, Reilly JA, Lysko PG, Henneberry, RC (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res* 451: 205-212
46. Novikoff AB (1973) Lysosomes : A personal account. In: Lysosomes and Storage Diseases, Hers HG, van Hoof F (eds.), Chapter 1, pp. 2-41, Academic Press: New York
47. Overly CC, Lee K, Berthiaume E, Hollenbeck PJ (1995) Quantitative measurement of intraorganelle pH in the endosomal-lysosomal pathway in neurons by using ratiometric imaging with pyranine. *Proc Natl Acad Sci USA* 92: 3156-3160
48. Palmer DN, Fearnley IM, Walker JE, Hall NA, Lake BD, Wolfe LS, Haltia M, Martinus RD, Jolly RD (1992) Mitochondrial ATP synthase subunit c storage in the ceroid lipofuscinoses (Batten disease). *Am J Med Genet* 42: 561-567
49. Parton RG, Dotti CG (1993) Cell biology of neuronal endocytosis. *J Neurosci Res* 36: 1-9
50. Parton RG, Simons K, Dotti CG (1992) Axonal and dendritic endocytic pathways in cultured neurons. *J Cell Biol* 119: 123-137
51. Pentchev PG, Comly ME, Kruth HS, Vanier MT, Wenger DA, Patel S, Brady RO (1985) A defect in cholesterol esterification in Niemann-Pick disease (type C) patients. *Proc Natl Acad Sci USA* 82: 8247-8251
52. Purpura DP, Suzuki K (1976) Distortion of neuronal geometry and formation of aberrant synapses in neuronal storage disease. *Brain Res* 116: 1-21
53. Rabin SJ, Mocchetti I (1995) GM1 ganglioside activates the high-affinity nerve growth factor receptor TRKA. *J Neurochem* 65: 347-354
54. Rider JA, Dawson G, Siakotos AN (1992) Perspective of biochemical research in the neuronal ceroid-lipofuscinosis. *Am J Med Genet* 42: 519-524
55. Sachs B (1887) On arrested cerebral development, with special reference to its cortical pathology. *J Nervous Ment Dis* 14: 541-553
56. Sachs B (1903) On amaurotic family idiocy. A disease chiefly of the gray matter of the central nervous system. *J Nervous Ment Dis* 30: 1-13
57. Sachs B, Strauss I (1910) The cell changes in amaurotic family idiocy. *J Exp Med* 12: 685-695
58. Sandhoff K, van Echten G (1994) Ganglioside metabolism: Enzymology, topology, and regulation. *Prog Brain Res* 101: 17-30
59. Sandhoff K, Klein A (1994) Intracellular trafficking of glycosphingolipids: Role of sphingolipid activator proteins in the topology of endocytosis and lysosomal digestion. *FEBS Lett* 346: 103-107
60. Sandhoff K, Kolter T (1996) Topology of glycosphingolipid degradation. *Trends Cell Biol* 6: 98-103
61. Sandhoff K, Schwarzmann G (1989) Dynamics of gangliosides in neuronal membranes. In: Progress in Zoology, Voume 37: Fundamentals of Memory Formation; Neuronal Plasticity and Brain Function., Rahmann H (ed.), pp. 229-239, VCH Publishers: New York
62. Schwarzmann G, Sandhoff K (1990) Metabolism and intracellular transport of glycosphingolipids. *Biochemistry* 29: 10865-10870
63. Scriver CR, Beaudet AL, Sly WS and Valle D (eds.) (1995) Lysosomal enzymes. The Metabolic and Molecular Bases of Inherited Disease, Chapters 76-92, pp. 2427-2879, McGraw-Hill: New York
64. Siegel DA, Walkley SU (1994) Growth of ectopic dendrites on cortical pyramidal neurons in neuronal storage diseases correlates with abnormal accumulation of GM2 ganglioside. *J Neurochem* 62: 1852-1862
65. Sleat DE, Donnelly, RJ, Lackland H, Liu C, Sohar I, Pullarkat RK, Lobel P (1997) Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid -lipofuscinosis. *Science* 277: 1802-1805
66. Smith RS (1980) The short term accumulation of axonally transported organelles in the region of localized lesions of single myelinated axons. *J Neurocytol* 9: 39-65
67. Suzuki K (1976) Neuronal storage diseases: A review. In: Progress in Neuropathology, Zimmerman HM (ed.), Volume 3, pp. 173-202, Grune & Straton: New York
68. Suzuki K (1994) Molecular genetics of Tay-Sachs and related disorders: a personal account. *J Neuropathol Exp Neurol* 53: 344-350
69. Swanson JA, Watts C (1995) Macropinocytosis. *Trends Cell Biol* 5: 424-428
70. Terry R, Weiss M (1963) Studies in Tay-Sachs disease II. Ultrastructure of the cerebrum. *J Neuropathol Exp Neurol* 22: 18-55
71. Tsukita S, Ishikawa H (1980) The movement of membranous organelles in axons: electron microscopic identification of anterogradely and retrogradely transported organelles. *J Cell Biol* 84: 513-530
72. van Echten G, Sandhoff K (1993) Ganglioside metabolism - enzymology, topology and regulation. *J Biol Chem* 268: 5341-5344
73. van Meer G, Burger KNJ (1992) Sphingolipid trafficking - sorted out? *Trends Cell Biol* 2: 332-337

74. Wada I, Lai WH, Posner BI, Bergeron JJM (1992) Association of the tyrosine phosphorylated epidermal growth factor receptor with a 55-kD tyrosine phosphorylated protein at the cell surface and in endosomes. *J Cell Biol* 16: 321-330

75. Walkley SU (1987) Further studies on ectopic dendrite growth and other geometrical distortions of neurons in feline GM1 gangliosidosis. *Neuroscience* 21: 313-331

76. Walkley SU (1988) Pathobiology of neuronal storage disease. *Int Rev Neurobiol* 29: 191-244

77. Walkley SU (1995) Pyramidal neurons with ectopic dendrites in storage diseases contain elevated levels of GM2 ganglioside. *Neuroscience* 68: 1027-1035

78. Walkley SU, Wurzelmann, S (1995) Alterations in synaptic connectivity in cerebral cortex in neuronal storage diseases. *Ment Retard Dev Disabilities Rev* 1: 183-192

79. Walkley SU, Baker HJ (1984) Sphingomyelin lipidosis in a cat. II. Golgi studies. *Acta Neuropathol (Berl)* 65: 138-144

80. Walkley SU, Baker HJ, Rattazzi MC (1990) Initiation and growth of ectopic neurites and meganeurites during postnatal brain development in ganglioside storage disease. *Dev Brain Res* 51: 167-178

81. Walkley SU, Baker HJ, Rattazzi MC, Haskins ME, Wu J-Y (1991) Neuroaxonal dystrophy in neuronal storage disorders: Evidence for major GABAergic neuron involvement. *J Neurol Sci* 104: 1-8

82. Walkley SU, Blakemore WF, Purpura DP (1982) Alterations in neuron morphology in feline mannosidosis: A Golgi study. *Acta Neuropathol (Berl)* 53: 75-79

83. Walkley SU, Haskins ME, Shull RM (1988) Alterations in neuron morphology in mucopolysaccharide storage disorders. *Acta Neuropathol (Berl)* 75: 611-620

84. Walkley SU, March PA, Schroeder CE, Wurzelmann S, Jolly RD (1995) Pathogenesis of brain dysfunction in Batten's disease. *Am J Med Genet* 57: 196-203

85. Walkley SU, Pierok AL (1986) Ferric ion-ferrocyanide staining in ganglioside storage disease establishes that meganeurites are of axon hillock origin and distinct from axonal spheroids. *Brain Res* 382: 379-386

86. Walkley SU, Siegel DA (1989) Comparative studies of the CNS in swainsonine-induced and inherited feline α-mannosidosis. In: Swainsonine and Related Glycosidase Inhibitors, James LF, Elbein AD, Molyneux RJ, Warren CD (eds.), pp. 57-75, Iowa State University Press: Ames

87. Walkley SU, Siegel DA, Dobrenis, K (1995) GM2 ganglioside and pyramidal neuron dendritogenesis. *Neurochem Res* 20: 1287-1299

88. Walkley SU, Siegel DA, Wurzelmann S (1988) Ectopic dendritogenesis and associated synapse formation in swainsonine-induced neuronal storage disease. *J Neurosci* 8: 445-457

89. Walkley SU, Thrall MA, Dobrenis K, Huang M, March P, Siegel D, Wurzelmann S (1994) Bone marrow transplantation corrects the enzyme defect of the central nervous system in a lysosomal storage disease. *Proc Natl Acad Sci USA* 91: 2970-2974

90. Walkley SU, Wurzelmann S, Purpura DP (1981) Ultrastructure of neurites and meganeurites of cortical pyramidal neurons in feline gangliosidosis as revealed by the combined Golgi-EM technique. *Brain Res* 211: 393-398

91. Walkley SU, Wurzelmann S, Rattazzi MC, Baker, HJ (1990) Distribution of ectopic neurite growth and other geometrical distortions of neurons in feline GM2 gangliosidosis. *Brain Res* 510: 63-73

92. Walkley SU, Wurzelmann S, Siegel DA (1987) Ectopic axon hillock-associated neurite growth is maintained in metabolically reversed swainsonine-induced neuronal storage disease. *Brain Res* 410: 89-96

93. Williams RS, Lott, IT, Ferrante RJ, Caviness VS (1977) The cellular pathology of neuronal ceroid-lipofuscinosis. *Arch Neurol* 34: 298-305

94. Wong-Riley MTT (1989) Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. *Trends Neurosci* 12: 94-101

95. Yates AJ (1986) Gangliosides in the nervous system during development and regeneration. *Neurochem Pathol* 5: 309-329

96. Yu RK (1994) Developmental regulation of ganglioside metabolism. *Prog Brain Res* 101: 31-44

97. Zeller CB, Marchase RB (1992) Gangliosides as modulators of cell function. *Am J Physiol* C1341-C1355

98. Zeman W, Donahue S (1963) Fine structure of the lipid bodies in juvenile amaurotic idiocy. *Acta Neuropath (Berl)* 3: 144-149